Interactive Effects of Metals and PAHs on Benthic Food Webs

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LONG-TERM GOALS

Our long-term goal is to understand how complex mixtures of contaminants influence benthic communities at the levels of microorganisms, microalgae, invertebrate grazers, and fish predators. In particular, we are interested in how contaminants influence foodweb interactions among these groups of organisms.

OBJECTIVES

Our research examines the interactive effects of metal (Cu, Cr, Cd, Hg, and Pb) and diesel-fuel contaminants on the benthic food web of a coastal salt marsh, the specific role that Cu plays in this suite of contaminants, and how hypoxia influences the manifestation of toxic effects. Specifically, we are examining how diesel and metal contaminants interact to influence the microbial (bacteria and microalgae), invertebrate, and juvenile fish components of the benthic community, and how their interactions influence trophic relationships among organisms. Previous studies have focused on either the ecotoxicological effects of metals *or* the effects of hydrocarbons, but essentially nothing is known

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about how these two classes of contaminants interact. The modes of toxicity of hydrocarbons and metals are quite different, and individually they may elicit different, sometimes opposite, ecological responses. Impacted field sediments, especially in harbors, are typically contaminated with both metals and hydrocarbons, and thus ecological impacts may be a consequence of their interactive effects. Our experimental approach to this problem will provide fundamental information on the ecological manifestations of metals-hydrocarbons interactions, and provide the basis for making ecologically sound decisions concerning appropriate bioremediation or mitigation strategies for contaminated field sites.

APPROACH

Experimental work is divided into two components: microcosm experiments to examine contaminant effects on benthic microbes and grazers, and laboratory experiments to examine contaminant effects on fish predation on benthic invertebrates. As proposed, two major microcosms experiments were performed in year 1 to examine responses of, and interactions between microbes and meiofauna when exposed to metals and diesel-metal combinations under normoxic conditions. Future microcosm experiments will determine the influence of hypoxia on contaminant effects, and the specific role of Cu in mixtures of contaminants. The influence of diesel-metal mixtures on fish predation on meiofauna, and bioaccumulation of metals by fish, will be examined in years 2 and 3.

The microcosm approach used is one that we developed to study the influence of contaminants on interactions between microbes and meiofauna of sediment food webs (e.g., Carman et al. 97). The impacts of diesel and metal contaminants are determined not merely by monitoring the abundance of various groups of organisms, but by determining their physiological responses and trophic interactions (*i.e.*, grazing rates). The microcosms (15.2 cm i.d.) represent minimally disturbed, natural assemblages of benthic organisms and the sediment in which they live. Experiments are well replicated (n=5 per treatment) and include a complete set of uncontaminated controls, which allows rigorous hypothesis testing.

Microcosms of marsh sediment were collected by hand from mudflats associated with the *Spartina alterniflora* saltmarsh near the LUMCON facility in Cocodrie, LA and treated with diesel and metal contaminants as generally described by Carman et al. (1997). Natural photoperiods and temperature were maintained. The following community responses were determined: bacterial biomass and community composition; microalgal abundance, community composition, and productivity; meiofaunal abundance and community composition; and meiofaunal grazing on microalgae. Microtox assays will also be performed as a standard reference for sediment toxicity. In experiments involving diesel contamination, bacterial metabolism of PAH was also assayed. Hydrocarbon (PAH and aliphatic), and bulk sediment and pore-water metal concentrations, as well as Cu speciation were also determined.

WORK COMPLETED

In November, 1998 we performed a microcosm experiment to specifically examine the influence of a metal mixture on the benthic community. Sediments were contaminated with a mixture of known concentrations of Navy-relevant metals - Cu, Cr, Cd, Pb, and Hg - and concentrations were manipulated to simulate the relative abundances of metals in San Diego Harbor (SDH) (219 ppm Cu, 178 ppm Cr, 1 ppm Cd, 51 ppm Pb, 1 ppm Hg; Kennish 1997). In addition to control (uncontaminated microcosms), three metal concentrations were tested that bracket the concentrations noted above: "High" (10 x SDH), "Medium" (1.0 x SDH), and "Low" (0.1 x SDH). Effects were examined at days 0, 10, and 30

A second microcosm experiment to examine interactive effects of metals and diesel was performed in June, 1999. Results of the metals-only experiment were used to determine appropriate metal concentrations to be used in the diesel-metal interaction experiment. Diesel-metal interactions were examined at concentrations at which diesel and metals concentrations individually elicit major ecological responses, and at concentrations at which diesel or metals alone did not produce major ecological effects.

RESULTS

Metals Experiment:
Benthic microalgal biomass in general (Chl *a*), and diatom (fucoxanthin) and cynaobacteria (zeaxanthin) in particular increased significantly in medium- and high-metal treatments. In contrast to previous work with diesel-contaminated sediments, however, the increased biomass was not present as a distinct surface film of algae. Algal biomass associated with airstones (used to aerate overlying water in microcosms), however, exhibited a dose-dependent *decrease* in biomass. Greater sensitivity of airstone vs. benthic microalgae, and the lack of algal biofilm on sediment surfaces may reflect greater toxicity of dissolved relative to sediment-associated metals. Benthic algal productivity was not significantly influenced by metal treatments, indicating that benthic algae in metal-contaminated sediments were viable, and not dead or moribund.

Multivariate analysis of meiofaunal community structure revealed that the high-metals community differed significantly from all other treatments because of lower abundances of copepods, copepod nauplii, nematodes and polychaetes. Polychaetes were the most sensitive group, and were negatively impacted in both medium- and high-metal treatments. In contrast to polychaetes, copepod and nematode abundances were enhanced (though not significantly) in medium-metal treatments. Ostracod abundance was not significantly influenced by metal contamination. The principal grazers on microalgae were copepods, polychaetes, and ostracods. Grazing on microalgae was significantly reduced in high-metal treatments, primarily due to reduced abundances of polychaetes and copepods.

In collaboration with D.C. White and S. Macnaughton (Univ. of Tennessee), microbial community structure was examined using phospholipid fatty acid (PLFA) analysis and denaturing gel gradient electrophoresis (DGGE) of rRNA fragments. Metal treatment did not significantly influence total microbial biomass, but did influence microbial community composition. In particular, high-metal treatments increased the trans/cis PLFA ratio, indicating increased microbial stress, and increased the iso/anteiso PLFA ratio, indicating an increase in anaerobic Gram-negative bacteria. DGGE analysis revealed an enhanced abundance of a diatom (*Amphora* sp.) in medium- and high-metal treatments.

Diesel/Metals Interaction Experiment:
Sample processing from this experiment is still in progress, but data that we have thus far indicate that diesel-metal combinations have toxic effects that differ qualitatively from the effects of either class of contaminant alone. As observed in previous work (e.g., Carman et al. 1997), diesel contamination led to high mortality to crustacean grazers and the formation of benthic algal blooms. High metal-diesel combinations produced a synergistic effect in which algal blooms were completely eliminated, and algal productivity was significantly lower than in the presence of either metals or diesel alone. Individually, both high-metals and high-diesel concentrations dramatically reduced copepod abundance (Fig. 1). The effect was even more pronounced when metals and diesel fuel were present simultaneously. Neither low metals nor low diesel significantly reduced copepod abundance, but, in combination, copepod abundance was reduced approximately six-fold. We also observed that metals-

diesel combinations significantly slowed the decomposition rate of copepod carcasses, implying that bacteria responsible for decomposition were negatively impacted.

Macroscopic and microscopic examination of sediment surfaces also indicated dramatic influences of contaminants on sediment biota. Dense algal biofilms were present on the surface of diesel-contaminated sediments. No algal biofilm was present on the surface of metal-contaminated sediments, and biogenic structures (pits, fecal mounds, etc.) were rare. When high-metal concentrations were combined with diesel, sediment surfaces appeared sterile, with no signs of biological activity. Microscopic analysis revealed that diatom diversity and abundance was lowest in metal and metal-diesel contaminated sediments.

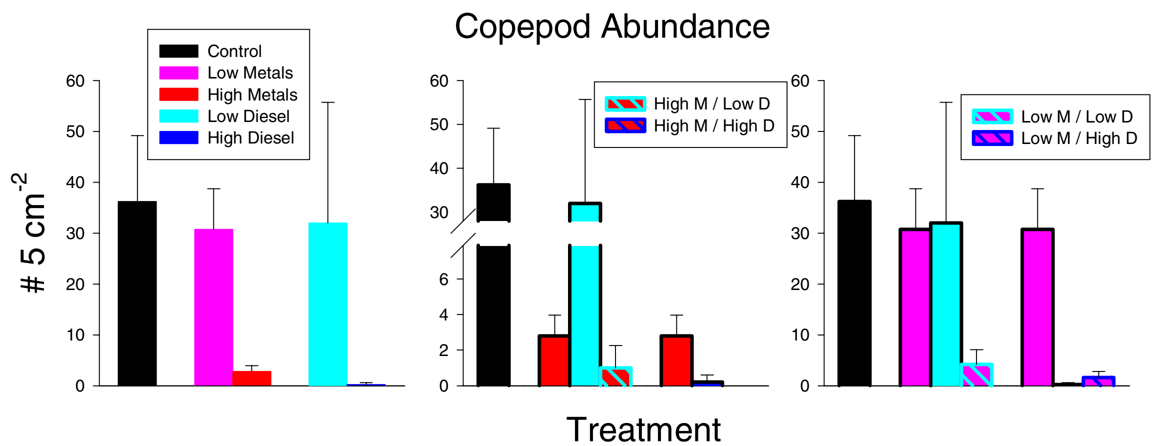

Fig. 1: Effects of metals and diesel alone (left panel), 10x SDH metal concentrations in combination with diesel (middle panel), and 1x SDS metal concentrations in combination with diesel (right panel). No copepods were detected in the High Metal/High Diesel combination. Note the significant reduction in copepod abundance in the Low Metal/Low Diesel combination in comparison to either contaminant individually.

IMPACT/APPLICATIONS

Collectively, results of the metals experiment indicate: (1) changes in the meiofaunal community with high-metal exposure due to decreases in copepods, copepod nauplii, nematodes and polychaetes, (2) decreased meiofaunal grazing impact in high metals, primarily due to decreases in meiofaunal abundance, (3) changes in the bacterial community in high metals, indicating more stress and a shift toward anaerobes, and (4) enhanced microalgal (diatom and cynaobacterial) biomass in medium- and high-metal treatments. Enhanced algal biomass may partially be a response to reduced grazing pressure, but enhancements of both meiofaunal abundance and microalgal biomass in medium treatments suggest a stimulatory effect at lower concentrations. Thus, while high (10x SDH) metal concentrations are toxic, medium (1x SDH) concentrations may have stimulatory effects on microalgae and meiofauna. Based on these results, we decided to use high and medium concentrations in the diesel-metals interaction experiment.

Preliminary results from the diesel-metals interaction experiment indicate that combinations of diesel and metals produce toxic effects that are not simply additive. It appears that sediments contaminated

with high concentrations of both metals and diesel are substantially more toxic to meiofauna, microalgae, and bacteria than when either class of contaminants are present individually. Further, toxicological effects on copepods were observed at lower concentrations of combined metals and diesel, neither of which individually produced substantial toxicological effects. This result indicates that the toxicity of natural sediments containing a complex mixture of metals and hydrocarbons may not be accurately assessed by simply summing the toxicological effects of individual components. The toxicological mechanism(s) for these synergistic effects are not yet known

TRANSITIONS

Results of our experimental work are being used in an on-going collaboration with Roger Nisbet (UCSB) and colleagues to develop predictive models of the effects of contaminants on benthic communities. We are also collaborating with D.C. White (Univ. Tenn.) to examine effects on contaminants on microbial community structure.

RELATED PROJECTS

1 - "How does produced water cause a reduction in the genetic diversity of harpacticoid copepods?" Minerals Management Service (J. Fleeger, PI and D. Foltz, Co-PI), $241,000, 1998-01. (An investigation into the influence of PAH's on genetic diversity of copepods.)

2 - "Effects of epibionts on harpacticoid copepods in a Louisiana saltmarsh". K.R. Carman PI; G. Puckett Co-PI. Environmental Protection Agency, $52,722. 1998-2000. (Investigation of the influence of protozoan epibionts on copepod susceptibility to contaminants.)

3 – National Center for Ecological Analysis and Synthesis (NCEAS) working group. Roger Nisbet (UCSB) is heading a working group to develop a model of contaminant effects on the microalgae-meiofauna food web that is the focus of our past and present ONR experimental work.

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